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Sub-Soiling and Genotype Selection Improves *Populus* Productivity Grown on a North Carolina Sandy Soil

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Abstract: This study reports the stem volume of 10 *Populus* genotypes in a randomized split-plot design with different tillage treatments (disking *versus* sub-soiling) after two years of growth. Height, diameter at breast height (DBH), stem aboveground volume index, survival, *Melampsora* rust resistance, leaf area index (LAI), chlorophyll content, and foliar nitrogen concentration (Foliar N) were measured to identify how tillage treatments might alter poplar growth. Stem volume index and LAI were positively correlated and differed significantly among tillage treatments, taxa, and genotypes. *Melampsora* rust resistance was also positively correlated with volume index, but significant differences were only detected among taxa and genotypes. Foliar N and chlorophyll did not correlate to stem volume for genotypes or tillage treatments. Overall, sub-soiling yielded 37% more estimated volume compared to disking. Within the sub-soiled treatments, four genotypes (140, 176, 185, and 356) had high survival (>80%) and produced substantial stem volume (>32 $dm^3 \cdot tree^{-1}$). These findings show that tillage practices do impact poplar stem volumes after two years and that sub-soiling improves productivity for poplar short rotation woody crops on loamy fine-sandy soils.

Keywords: disking; short rotation woody crops; tillage; site preparation; volume index; *Melampsora* rust

1. Introduction

International and domestic policies mandate increased reliance on forest resources to decrease greenhouse gas emissions and global dependence on fossil fuels. In the European Union (E.U.), the renewable energy directive has mandated that renewables represent 20% of total energy by 2020, and many participating countries are importing wood pellets from the southeastern U.S.A. to supplement their portfolios [1,2]. In the U.S.A., the 2010 Renewable Fuel Standard mandates that the volume of biofuel blended into transportation fuels increase from 9 billion gallons in 2008 to 36 billion gallons by 2022 [3]. Given these policies, a large amount of land (between 33 and 51 million hectares (ha)) is needed to grow dedicated biomass in highly productive plantations [4,5]. The southeastern U.S.A. represents a promising region with over 80 million ha in timber production and a large area of marginal and abandoned lands available for dedicated biomass production [6–11]. The U.S. Department of Energy estimates that purpose-grown woody crops with productivities between 8 and 10 green Mg $ha^{-1} \cdot year^{-1}$ will be required at a minimum if demand for wood pellets and cellulosic ethanol is to be met [7].

Poplars (*Populus* spp.) are one option for purpose-grown woody crops or short rotation woody crops (SRWC) [12,13]. Poplar SRWC have estimated stem volume indices ranging from 2.25 to 29.3 Mg $ha^{-1} \cdot year^{-1}$ in the Pacific Northwest [14–17], 0.4 to 24.5 Mg $ha^{-1} \cdot year^{-1}$ in the North

Central region [18–20], and 0.1 to 24.1 Mg ha^{-1} year^{-1} in the Northeast [21]. Poplar SRWC studies for the southeastern U.S.A. are limited to the Mississippi Delta [22–26] or the U.S. Department of Energy Savannah River Site in South Carolina [27–32]. Outside of these areas, poplar trials are underway in Kentucky [33], Georgia [34], and North Carolina [35,36], but these efforts do not address whether different tillage practices yield significantly greater biomass for commercially-available poplar genotypes.

Though *Populus* plantations are known to yield greater biomass in tilled soils *versus* non-tilled soils, the impact of different tillage practices remains largely unexplored [37,38]. Common practices include disking, harrowing, bedding, sub-soiling, or a combination of these techniques [39,40]. In *Pinus* spp. (pine) plantations, disking has the potential to reduce root competition, improve water infiltration, and increase nutrient availability. Similarly, sub-soiling may increase infiltration rates and improve root growth in compacted soils [39]. Whether these tillage practices affect poplar productivity remains unclear. Because equipment purchases represent a substantial upfront cost to landowners [41], there is a need to evaluate the tradeoffs between tillage practices before successful poplar SRWC deployment can occur throughout the southeastern U.S.A.

This study assessed the effect of two tillage practices (disking *versus* sub-soiling) on 10 *Populus* genotypes representing three taxa after two years of growth using a completely randomized split-plot design on loamy fine-sandy soils in North Carolina. The effects of tillage treatments were quantified for each genotype using the following metrics: tree stem volume index, leaf area index (LAI), foliar nitrogen concentration (foliar N), chlorophyll content, and evidence of disease. These traits were additionally evaluated to identify significant correlations among the assessed characteristics and stem volume. Greater foliar N often correlates to increased poplar SRWC productivity [16,18,23], and chlorophyll content can be utilized as indicator of foliar N [42–44]. However, assessments are limited for poplar genotypes. Correlations between chlorophyll content and foliar N have been documented for other tree species such as sugar maple (*Acer saccharum* Marsh.) [42], American sycamore (*Platanus occidentalis* L.) [43], sweetgum (*Liquidambar styraciflua* L.) [43], green ash (*Fraxinus pennsylvanica* Marsh.) [43], swamp cottonwood (*Populus heterophylla* L.) [43], shining gum (*Eucalyptus nitins* Deane & Maiden) [44], and blue gum (*Eucalyptus globulus* Labill.) [44]. Similarly, correlations between stem volume with phenotypic traits like leaf area and disease resistance are also limited for poplar SRWC grown in the southeastern U.S.A. Leaf area [45–47] and the presence or absence (resistance) of disease, such as *Melampsora* spp. leaf rust, have been correlated to poplar SRWC productivity elsewhere [19,28,34,48,49], but modern commercially-available genotypes have not been well documented. These evaluations may provide useful insight to explaining how maximum plantation productivity can be achieved as poplar SRWC expands in the southeastern U.S.A.

2. Materials and Methods

2.1. Site Description, Design, and Establishment

The poplar SRWC trial was installed on a former cornfield located in Wallace, NC, U.S.A. (34°45′N, 78°50′W). The climate is humid subtropical with annual precipitation of 1617 mm in 2013 and 899 mm in 2014. Site elevation is 16 m above mean sea level. The site soils are Noboco loamy fine sand with a pH of 6.56 ± 0.31, a cation exchange capacity of 7.39 ± 1.65 meq/100 g, 11.1 ± 2.89 kg ha^{-1} nitrate, 1,048 kg· ha^{-1} of phosphorous, and 242 kg· ha^{-1} of potassium. The soils are 87% sand, 6% silt and 7% clay.

In November 2012, a pre-emergent herbicide mixture of 4.67 L· ha^{-1} of 37.4% pendimethalin (Pendulum®, Green Resource LLC, Colfax, NC, USA) and 0.29 L· ha^{-1} of 70% imazquin (Image® 70DG, Green Resource LLC, Colfax, NC, USA) was applied. Subsequently, the site was divided into three blocks then subdivided into two tillage whole plots that were either disked or sub-soiled for a split-plot experimental design. Within each whole plot, 10 subplots were randomly assigned either a pure *Populus deltoides* (*P. deltoides*) taxa (140, 176, 356, 373), a *Populus trichocarpa* × *Populus deltoides*

(*P. trichocarpa* × *P. deltoides*) taxa (185, 187, 188, 229, 339), or a *Populus deltoides* × *Populus maximowiczii* (*P. deltoides* × *P. maximowiczii*) taxon (230). All tree materials were provided by ArborGen Inc. (Ridgeville, SC, USA) as 25 to 30 cm dormant cuttings. In February 2013, the site was treated with 41% glyphosate to remove weed competition. *Populus* cuttings were soaked for 24 to 48 h in water to enhance root initiation. Each subplot was planted at a 2.4 m × 2.7 m spacing (1543 trees hectare^{-1}) in a 4 × 4 tree layout. Two border rows of assorted *Populus* cuttings and loblolly pine (*Pinus taeda* [*L.*]) seedlings were planted on the perimeter of each whole-plot to reduce potential border effects [50]. After planting, the site was mowed monthly to minimize weed competition.

2.2. Data Collection for Tree Growth and *Melampsora* spp. Rust Resistance Index

All trees were inventoried for survival, growth, and *Melampsora* spp. rust presence in November 2014. The stem(s) for each tree were measured for height to apical bud, diameter at breast height (DBH), and survival. *Melampsora* rust was scored as a modified Schreinder index [51] where the percentage of leaves were scored on a scale of 0 to 4 (where 0 indicated 100% of foliage had observable rust; 1, 75%; 2, 50%; 3, 25%; and 4, no evidence of *Melampsora* spp. rust). To estimate aboveground tree volume, a volume index was calculated using $V = h \times d^2$, where V is volume index in cubic decimeters, h is height in decimeters, and d is DBH in decimeters [52]. For multiple stems, volume index was estimated for each individual stem and then summed for a total. Measurements were duplicated for at least 5% of all trees to evaluate precision of field data collection and are provided in the Supplementary Material (Table S1).

2.3. LAI, Chlorophyll Content, and Foliar N

LAI, chlorophyll content, and foliar N were evaluated in September 2014 before senescence was observed in two randomly-selected blocks. LAI was assessed using a LAI 2000 Plant Canopy Analyzer (LiCor, Lincoln, NE, U.S.A.) by comparison of above and below canopy readings. Eight observations were collected near the trunk of each tree within each 16-tree subplot to create a composite LAI value for the sub-plot. Chlorophyll content and foliar N were determined by removing and compositing 10 to 15 green leaves from two interior trees in each subplot. Sampling was carried out in the first two weeks of September when foliar nutrients are considered the most stable in poplars [53]. Leaves were analyzed for chlorophyll content using a chlorophyll meter from atLeaf+ (Wilmington, DE, USA), and foliar tissue was analyzed for percent nitrogen composition by Waters Agricultural Laboratories (Warsaw, NC, USA). Measurements were duplicated for at least 10% of collected samples to evaluate precision of field data collection (Table S1).

2.4. Statistical Analyses

Height (m), DBH (cm), volume index (dm^3), LAI, *Melampsora* rust resistance, chlorophyll content (mg· g^{-1}), and foliar N (%) were analyzed using analyses of variance (ANOVA, Proc Glimmix, SAS Inc., Cary, NC) assuming a split-plot design. To assess the significance of fixed effects, the following model was applied:

$$Y_{ijklm} = \mu + B_i + T_j + P_k + P(C)_{kl} + TP(C)_{jkl} + BTP(C)_{ijkl} + \varepsilon_{ijklm}$$

where Y_{ijklm} is the response variable to be analyzed, μ is the overall mean, B_i is the random effect of the ith block, T_j is the fixed effect of the jth tillage treatment, P_k is the fixed effect of the kth taxon, $P(C)_{kl}$ is the nested fixed effect of the lth genotype within the kth taxon, $TP(C)_{jkl}$ is the fixed interaction between the jth tillage treatment and lth genotype within the kth taxon, $BTP(C)_{ijkl}$ is the random interaction between the ith block and jth tillage treatment with lth genotype within the kth taxon, and ε_{ijklm} is the pooled residual error.

3. Results

3.1. Tillage and Genotype Selection

Tillage significantly affected height, DBH, and volume index (Table 1). Most poplar genotypes grown in the sub-soiled treatment had significantly greater heights and diameters than those genotypes grown in disked soils (Table S2 contains summary statistics for all growth measurements). Two genotypes (230 and 187) produced more volume in the disked treatment, but that difference was not significant ($p > 0.10$). Survival was greater in the sub-soiled plots (88%) compared to the disked plots (80%), but this difference was marginal compared to the difference in total estimated aboveground volume. Disked treatments produced 7583 dm^3, whereas the sub-soiled tillage treatments produced 37% more volume, a total estimated volume of 10,216 dm^3.

Taxa and genotype selection were also significant factors in achieving maximum productivity (Table 1). Three genotypes (187, 188, and 339) had substantially lower mean volume index (<15 $dm^3 \cdot tree^{-1}$) compared to other genotypes and may not be appropriate for larger poplar SRWC plantations (Figure 1). In contrast, four genotypes (140, 176, 185, and 356) grown in the sub-soiled treatment demonstrated potential to meet demand for cellulosic ethanol and wood pellets (Figure 1). These genotypes had > 80% survival and produced $\geqslant$ 32 $dm^3 \cdot tree^{-1}$. When a wood density of 0.37 $g \cdot cm^{-3}$ was assumed for two-year old poplars [54], plot productivities of these genotypes' would exceed 8.0 green Mg $ha^{-1} \cdot year^{-1}$. Thus, tillage does alter poplar productivity among genotypes.

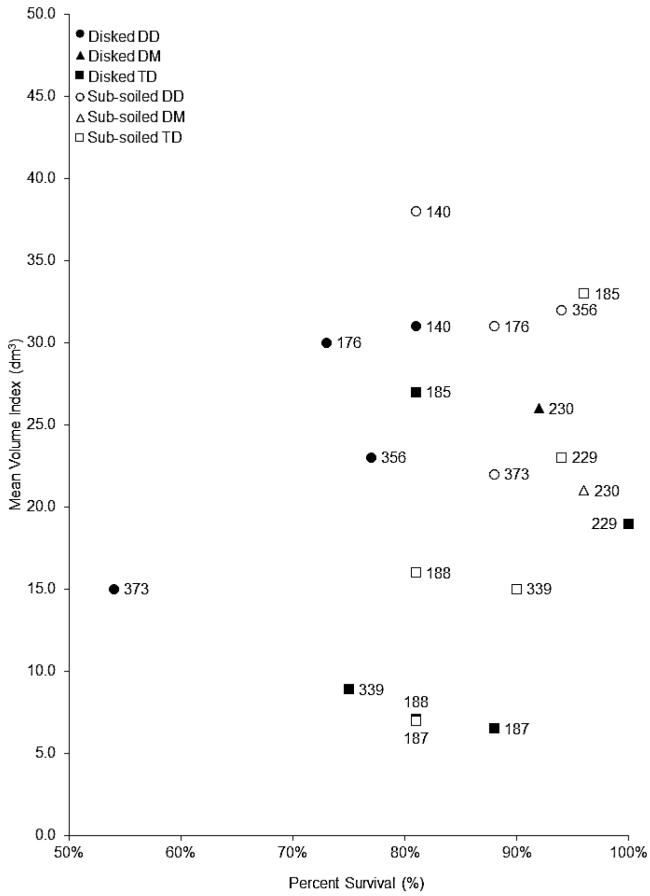

Figure 1. Cross-plot of mean volume index (dm^3) against percent survival for *Populus* genotypes in disked and sub-soiled plots. Open-faced symbols represent sub-soiled genotype plots and close-faced symbols represent disked genotype plots. Circles (O) represent *P. deltoides* genotypes abbreviated as DD; triangles (Δ) represent *P. deltoides* × *P. maximowiczii* genotypes abbreviated as DM; and squares (□) represent *P. trichocarpa* × *P. deltoides* genotypes abbreviated as TD.

Table 1. Probability values from analyses of variance for height, diameter at breast height (DBH), volume index, leaf area index (LAI), *Melampsora* rust resistance index, foliar nitrogen concentration (Foliar N), and chlorophyll content comparing ten poplars under two soil tillage treatments. Significant probability values ($p < 0.05$) are in bold.

Source of Variation	Height (m)	DBH (cm)	Volume Index (dm^3)	LAI	*Melampsora* Rust Resistance Index	Foliar N (%)	Chlorophyll Content ($mg{\cdot}g^{-1}$)
	p-Value	*p*-Value	*p*-Value	*p*-Value	*p*-Value	*p*-Value	*p*-Value
Soil Tillage	**0.026**	**0.020**	**0.044**	**0.040**	0.089	0.30	**0.027**
Taxa	**<0.0001**	**<0.0001**	**0.0003**	**0.0013**	**<0.0001**	0.27	**0.023**
Taxa(Genotype)	**0.0002**	**0.0005**	**0.0030**	**0.017**	**<0.0001**	0.060	0.19
Soil Tillage × Taxa(Genotype)	0.86	0.80	1.0	0.96	0.55	0.36	0.79

3.2. Chlorophyll Content, Foliar N, and Volume Index

Though significant differences were detected among genotypes for foliar N and volume index (Table 2), significant correlations were not observed between the two measured parameters ($R^2 = 0.0002$). Similarly, significant correlations were not observed between chlorophyll content and foliar N ($R^2 = 0.0001$) or between chlorophyll content and volume index ($R^2 = 0.0002$) (Figure S1 contains scatterplots for chlorophyll content, foliar N, and volume index; Table S3 contains summary statistics for measured foliar nutrient concentrations). Ranking genotypes for foliar N, chlorophyll content, and volume index revealed that genotypes with higher volume ranks tended to rank lower in foliar N (Table 2) except for genotype 140. Otherwise, trends could not be detected among the measured parameters. Thus, there were no relationships among chlorophyll content, foliar N, and volume index.

Table 2. Rankings for mean foliar nitrogen concentration (Foliar N), mean chlorophyll content, and mean volume index for *Populus* genotypes. Superscript letters in the rank columns denote significant differences between mean values according to a Tukey–Kramer *post hoc* analysis. Ranks with the same letters do not differ significantly.

Taxa	Genotype	Foliar N Mean (1 SD)	Nitrogen Rank	Chlorophyll Content Mean (1 SD)	Chlorophyll Rank	Volume Index Mean (1 SD)	Volume Rank
		(%)		($mg{\cdot}g^{-1}$)		(dm^3)	
DD	140	2.87% (0.34)	3^{BC}	0.034 (0.005)	2^{A}	34 (20)	1^{A}
DD	176	2.81% (0.42)	6^{C}	0.029 (0.003)	4^{A}	32 (24)	4^{ABC}
DD	356	2.68% (0.23)	9^{C}	0.022 (0.005)	1^{A}	30 (21)	3^{AB}
DD	373	2.82% (0.33)	4^{C}	0.032 (0.010)	5^{A}	19 (20)	7^{CDE}
DM	230	2.76% (0.33)	8^{C}	0.024 (0.007)	6^{A}	24 (18)	5^{ABC}
TD	185	2.81% (0.18)	5^{C}	0.030 (0.008)	10^{A}	33 (20)	2^{AB}
TD	187	3.22% (0.16)	1^{A}	0.024 (0.004)	9^{A}	19 (20)	10^{E}
TD	188	3.12% (0.44)	2^{AB}	0.025 (0.004)	8^{A}	29 (20)	9^{ED}
TD	229	2.64% (0.11)	10^{C}	0.028 (0.008)	7^{A}	20 (12)	6^{BCD}
TD	339	2.80% (0.18)	7^{C}	0.029 (0.008)	3^{A}	12 (10)	8^{ED}

TD: *P. trichocarpa* × *P. deltoides*; DD: *P. deltoides*; DM: *P. deltoides* × *P. maximowiczii*.

3.3. Melampsora Rust Resistance, LAI, and Volume Index

Significant correlations were detected amongst mean volume index, LAI, and *Melampsora* rust (Figure 2). According to an ANOVA, resistance to *Melampsora* rust resistance differed significantly among genotypes and taxa, but did not differ between tillage treatments (Table 1). A *post hoc* Tukey–Kramer analysis revealed that *P. deltoides* genotypes and the *P. deltoides* × *P. maximowiczii* genotype generally had higher rust resistance scores compared to *P. trichocarpa* × *P. deltoides* genotypes (Figure 2). The genotypes with the highest resistance were 176 and 356.

LAI also differed significantly among genotypes ($p = 0.017$) and taxa ($p = 0.0013$) as well as between tillage treatments ($p = 0.040$). Genotypes 185, 356, 140, and 176 demonstrated the highest

LAI in the sub-soiled tillage treatment (Table S4 contains summary statistics for LAI). These results demonstrate that LAI and disease resistance are well correlated with stem volume and selecting for these traits can improve establishment and early stem productivity in poplar SRWC grown in the southeastern U.S.A.

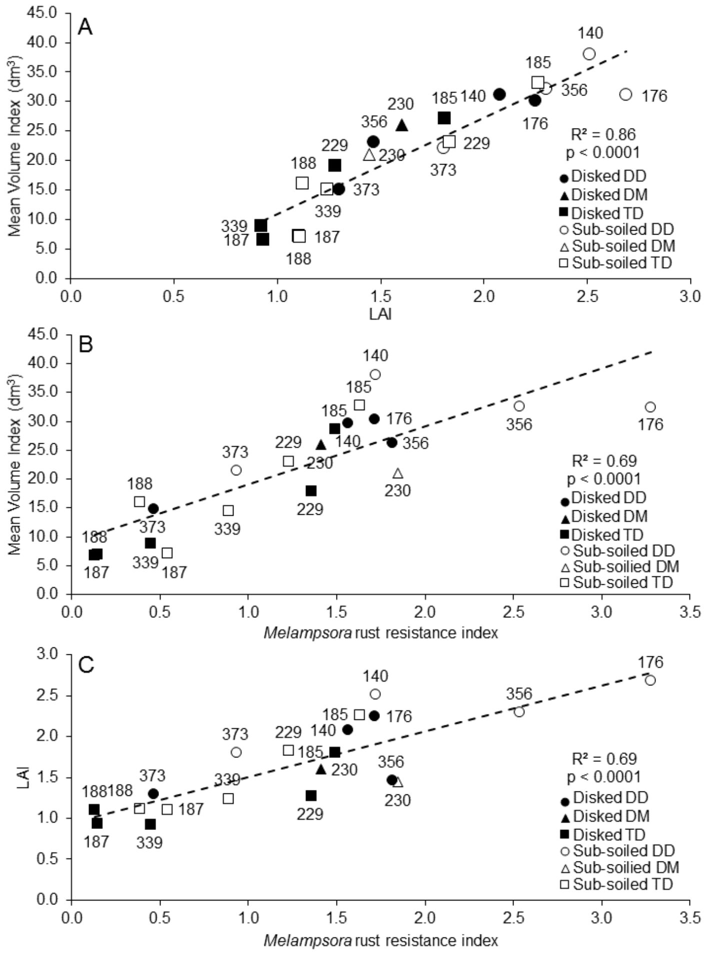

Figure 2. Cross-plots of (**A**) mean volume index (dm^3) *vs.* leaf area index (LAI); (**B**) mean volume index (dm^3) *vs.* *Melampsora* rust resistance index; and (**C**) LAI *vs.* *Melampsora* rust resistance index for *Populus* genotypes. Disked treatments are represented by close-faced symbols and sub-soiled treatments are represented by open-faced symbols. Circles (O) represent *P. deltoides* genotypes denoted as "DD", triangles (Δ) represent a *P. deltoides* × *P. maximowiczii* genotype denoted as "DM", and squares (□) represent *P. trichocarpa* × *P. deltoides* genotypes denoted as "TD". Trend lines are shown as dashed lines.

4. Discussion

4.1. Tillage and Genotype Selection

Few studies have evaluated the impact of tillage on *Populus* SRWC establishment and productivity. Morhart *et al.* [55] demonstrated that disking was preferred for improving stand survival to utilizing no tillage techniques and ley crops. In contrast, this study did not find a substantial difference in survival between the two tillage techniques. Additionally, this study found tillage practice to be a significant factor, resulting in a stand productivity difference of 37%. Genotype selection was also critical, which has been documented in other research efforts [28,29,32,36]. The improved performance of these *Populus* genotypes in the sub-soiled tillage treatment provides important insight to maximizing poplar SRWC productivity for larger plantations as disking is more common in the southeastern U.S.A. [24,27,29,30,33]. In other cases where sub-soiling has been utilized, relatively high productivities have been reported. For example, Heilman and Xie [16] reported on a sub-soiled plantation in the Pacific Northwest evaluating the performance of *P. trichocarpa* × *P. deltoides* under an array of fertilization treatments, and reported productivities ranging from 17.1 to 29.3 Mg $ha^{-1}\cdot year^{-1}$. Although productivities in this study were not as high as those reported by Heilman and Xie [16], the results do demonstrate that pairing careful genotype selection with soil tillage practice may be one key to achieving maximum productivity from *Populus* SRWC in multiple settings.

By combining these two experimental factors, three genotypes (140, 356, 185) grown in the sub-soiled plots demonstrated superior productivity compared to the other genotypes (Figure 1). The significance of sub-soiling and genotype selection in maximizing volume index highlights the importance of preliminary field trials for *Populus* SRWC. Though short field trials cannot capture long-term challenges like detrimental pest infestations or climate variability, they can be effective for identifying factors that improve woody biomass production and for eliminating genotypes that generally demonstrate poor performance [29,32]. This study identified several genotypes that would not be suitable for further deployment (187, 188, and 339) and determined that sub-soiling can improve early stand productivity. Though there is potential for the impact of sub-soiling on stand productivity to dissipate with rotation age [55], rapid early growth may be indicative of rankings later in rotation [32]. Therefore, monitoring tree productivity will continue at this research site. Given the large number of commercially-available genotypes [29], the variable performance of genotypes across a gradient of environmental conditions [20], and the limited number of field trials, this study demonstrates that there is a substantial need to identify silvicultural strategies to improve poplar SRWC establishment and growth in soils typical to the southeastern U.S.A.

4.2. Chlorophyll Content, Foliar N, and Volume Index

No correlation could be identified for *Populus* genotypes for any of the assessed parameters; however, there were significant differences in foliar N between genotypes (Table 2). This finding contradicts the findings of Moreau *et al.* [26] where a curvilinear relationship could be identified between foliar nitrogen and chlorophyll content for two *Populus* genotypes, and several other studies that correlated biomass production with foliar nitrogen [16,18,23]. A possible explanation for these contradictions is that the relationship between chlorophyll content, foliar nitrogen, and volume may only be correlated when foliar N is below critical concentrations. Blackmon [23], Hansen *et al.* [18], and Hielman and Xie [16] have suggested that the foliar N should be between 2.0% and 3.0% for optimal productivity. In this study, foliar N ranged from 2.18% to 4.05%; thus, all trees likely had sufficient nitrogen to synthesize chlorophyll and optimize productivity. This finding is supported by the lack of significant differences in chlorophyll content for the selected genotypes (Table 1). When nutrients do not limit productivity, other traits may be responsible for improved productivity such as disease incidence and/or resistance.

4.3. *Melampsora* Rust Resistance, LAI, and Volume Index

Significant correlations were identified for LAI, *Melampsora* rust index, and mean volume index (Figure 2). Significant differences were detected among genotypes and taxa means for both *Melampsora* rust resistance and LAI, suggesting that the expression of phenotypic traits like high leaf area and disease resistance is critical to poplar SRWC productivity. Though there is little debate on selecting for disease resistance [19,34,48] and high leaf area [56], there remains a need to identify modern commercially-available genotypes that demonstrate these traits [28]. This study identified four genotypes (185, 356, 140, and 176) with moderate *Melampsora* rust resistance and leaf area index. Selecting genotypes with high disease resistance is a strategy [37] that may result in decreased biomass yield later in rotation [19]. However, the results of this study suggest that genotypes with high *Melampsora* rust resistance have the potential to yield greater than eight green Mg $ha^{-1}\cdot year^{-1}$.

5. Conclusions

Poplar SRWC can potentially contribute biomass for alternative energy production in the southeastern U.S.A., but field screenings will be required to identify the best genotypes and assess silvicultural strategies that can improve productivity before large plantations can be deployed. This study found that proper soil tillage and genotype selection will be critical to achieve viable yields. Correlations between foliar nitrogen, chlorophyll content, and volume index were not observed perhaps due to adequate soil fertility, and suggests that chlorophyll meters are only effective in nutrient limited environments. Field assessments of phenotypic traits like high leaf area and *Melampsora* rust resistance may be more definitive in identifying highly productive *Populus* genotypes grown in nutrient-sufficient soils. This finding is important as more poplar SRWC are installed to meet the increasing demand for woody biomass markets.

Supplementary Materials: Supplementary materials are available online at http://www.mdpi.com/1999-4907/7/4/74/s1.

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Conflicts of Interest: The authors declare no conflict of interest.

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